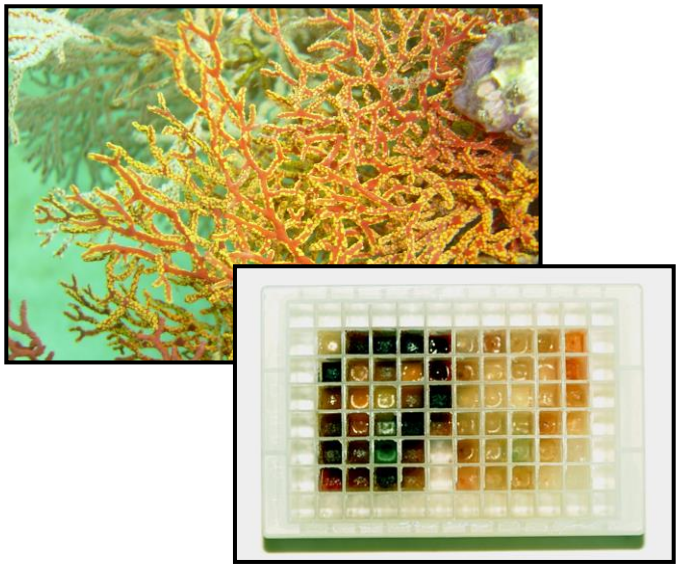
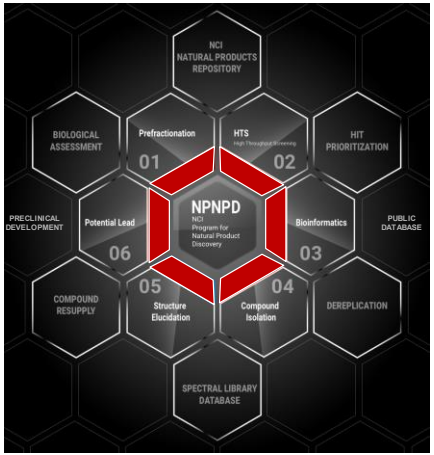
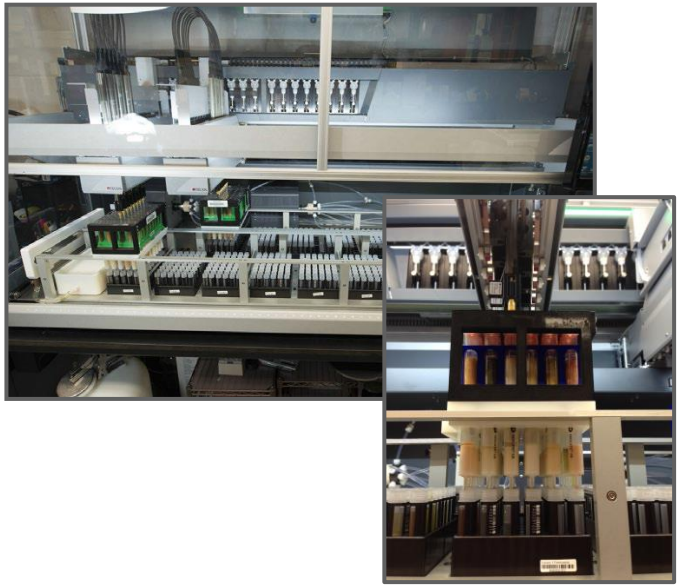
The NCI Program for Natural Product Discovery



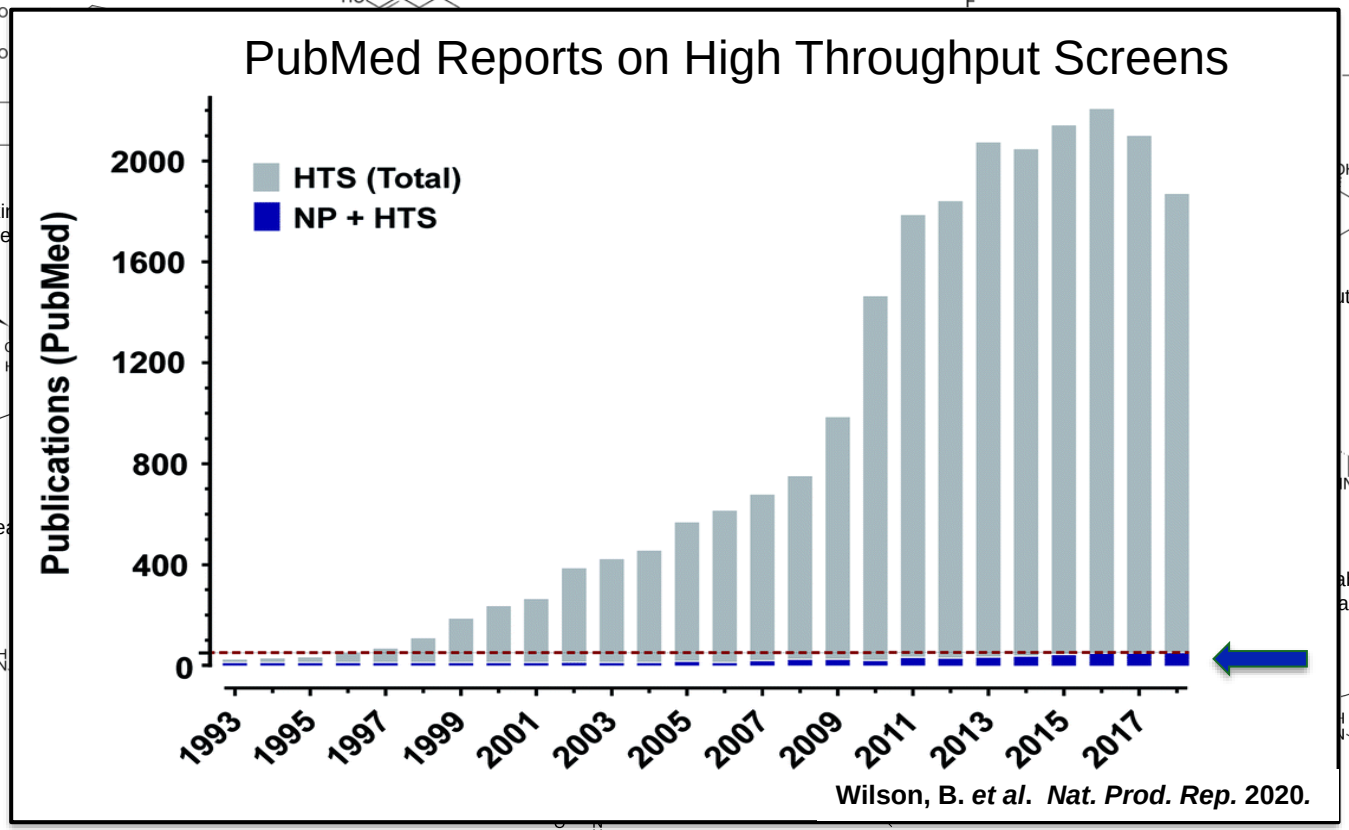




Barry R. O'Keefe

Director, Molecular Targets Program, Center For Cancer Research, and Chief, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, USA

Natural Product Drugs and High Throughput Screening



Omacetaxi
Chronic mye

Eribulin
Metastatic bre
cancer

Mifamurtide
Osteosarcoma

Brentuximab,
Hodgkin lymphoma

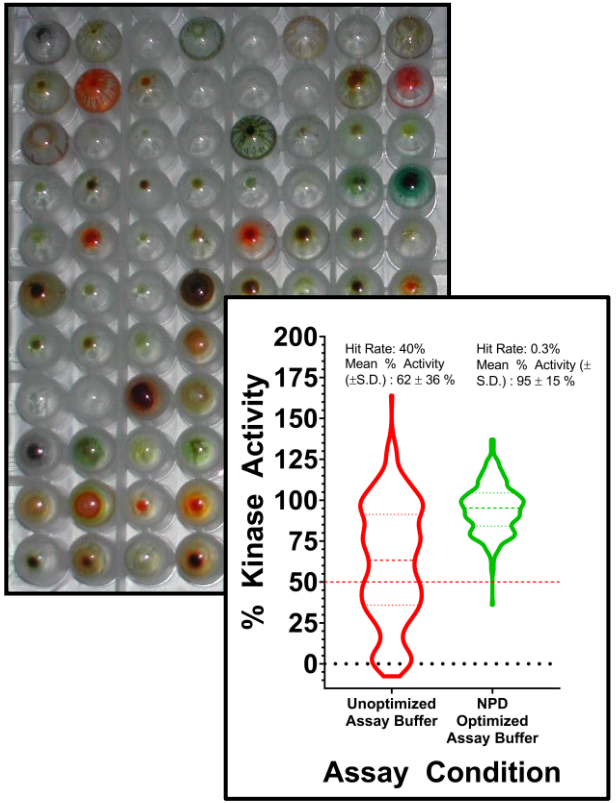
Plitidepsoin
ute lymphoblastic
leukemia

abepilone
ast Cancer

Carfilzomib
Multiple myeloma

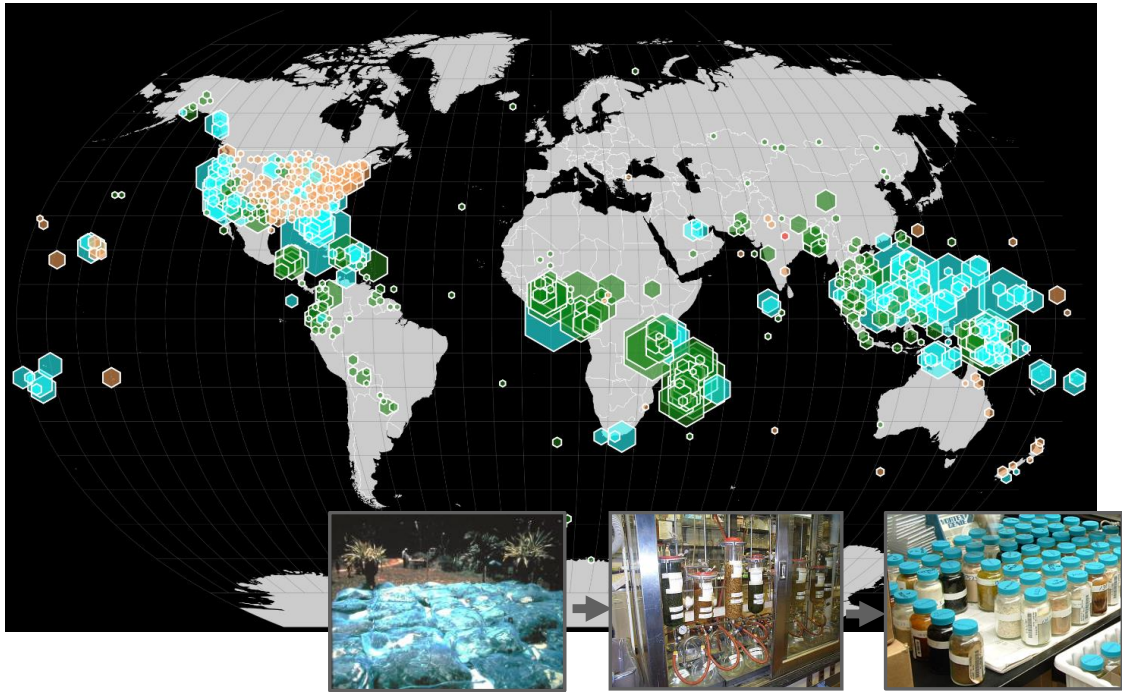
Why Have Natural Products Not Been Included in HTS?

- **Natural product extracts are difficult to screen in their crude form**
  - Cytotoxicity complicates cell-based assay systems
  - Common “nuisance” compounds complicate cell-free assay systems
- **Extracts contains numerous compounds at different concentrations**
  - Selecting appropriate test concentrations often a trade-off between sensitivity and high hit rates
- **Purification and structure elucidation of active compounds is time consuming and does not mesh well with HTS screening schedules**
  - History of long-term, low-throughput bioassay-guided fractionation
  - Results in slow process that increases overall costs for HTS
- **Need to address these challenges to efficiently access the unique chemical diversity in natural products**



NCI Natural Product Repository

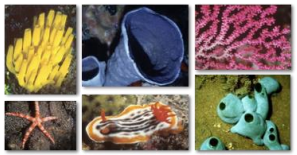
The NCI has one of the world's largest, most diverse collections of natural product extracts (>230,000 crude extracts).



Plant Extract Library

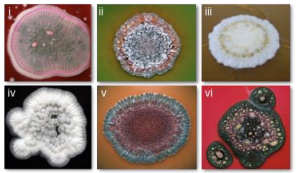
- **~161,000** extracts (organic + aqueous)
- **~44,000** plants, including 81,400 raw materials (leaves, roots, fruit, etc.) collected from Africa and Madagascar; North, Central and South America; and Southeast Asia.

Marine Extract Library



- **~41,000** extracts (organic + aqueous)
- **~20,500** organisms collected from the Indo-Pacific region.

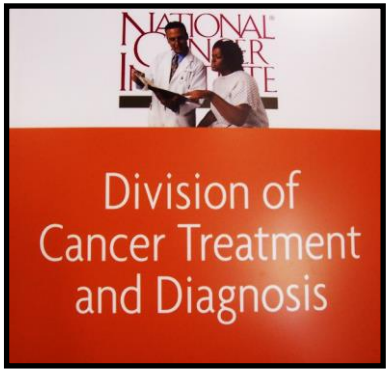
Microbial Extract Library

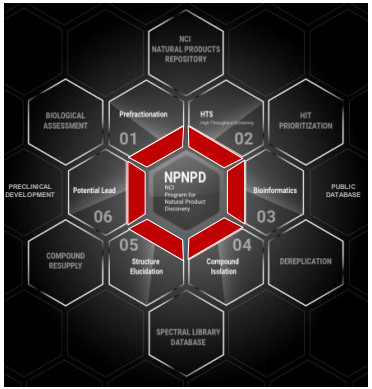


- **~30,000** extracts (organic + aqueous)
- **~26,000** organisms collected from US
- **New Collection**: 20,000 Fungal strains from USA (Univ. of Oklahoma)

NCI Program for Natural Products Discovery

The NCI Program for Natural Products Discovery (NPNPD) is a joint effort of the Division of Cancer Treatment and Diagnosis and the Center for Cancer Research.





The NPNPD is designed to facilitate both intramural and extramural research and address current challenges in natural product-based drug discovery.

The NPNPD is funded by the Cancer Moonshot Program.

NPNPD Cancer Moonshot Project Specific Aims

Aim 1. Create new technologies to build an enhanced NP pre-fractionated library amenable to modern high-throughput targeted screening programs.

Aim 2. Expand the chemical diversity available to the public from culturable microorganisms with new methods and libraries.

Aim 3. Provide the pre-fractionated library to screening centers worldwide to accelerate drug discovery.

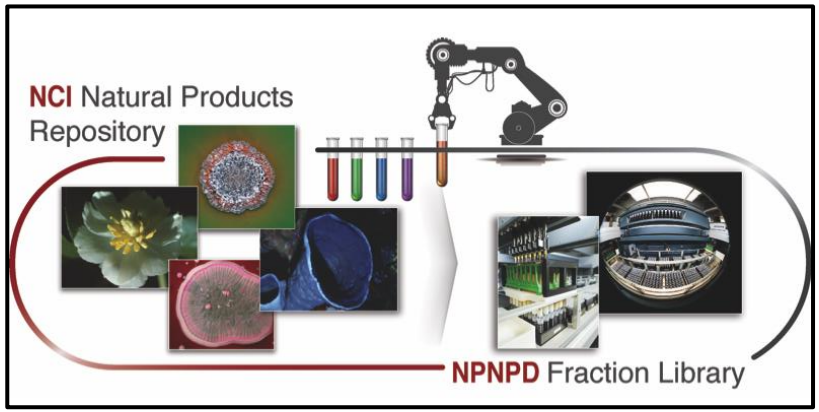
Aim 4. Encourage high throughput screening support for researchers to enable targeted discovery efforts.

Aim 5. Provide faster analytical resources (isolation, structure elucidation, re-supply) to expedite translational pipelines.

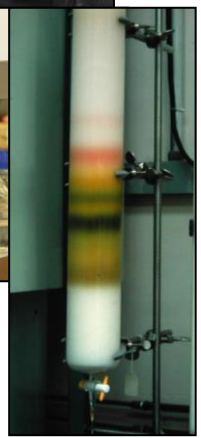
Aim 6. Establish a public database and bioinformatics platform to broaden input and expand impact.

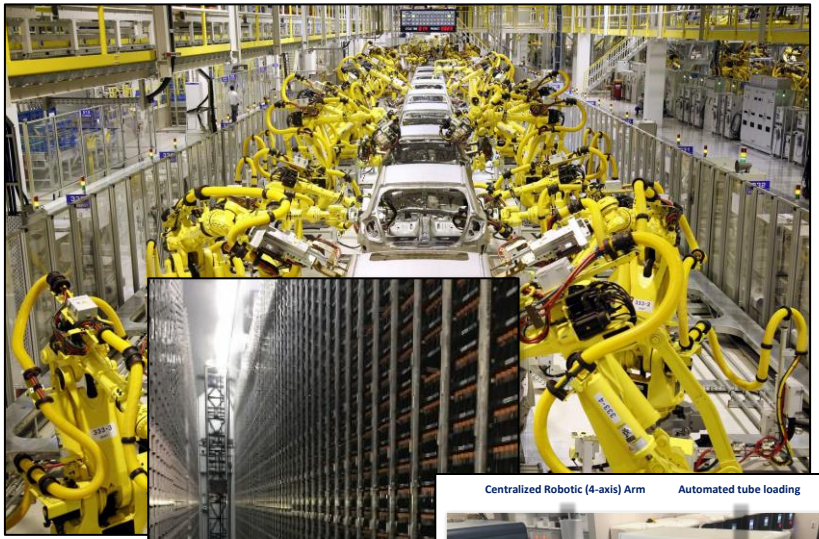
Pre-fractionation Plans

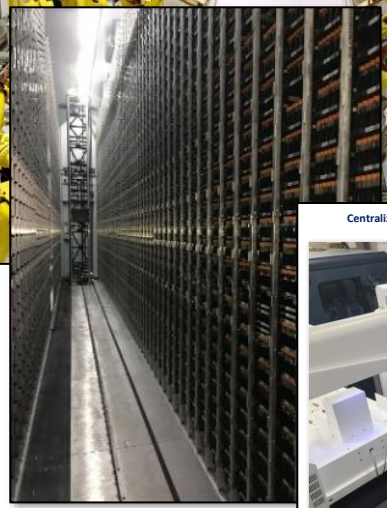
- Create a library of ~1,000,000 semi-pure natural product fractions more amenable to modern screening technologies
- Supply this library of chemical diversity to researchers for free
- Open use of the library to all screening labs, against all disease targets
- Use the pre-fractionated library to improve the efficiency of both high throughput screening and subsequent chemistry efforts
- Practical considerations:
  - Need to produce ~150,000 fractions per year
  - Sufficient mass to support screening programs for 10 yrs
  - Storage must allow for rapid automated access
  - Fractions must be plated in 384-well plates
  - Fractions must have a defined weight



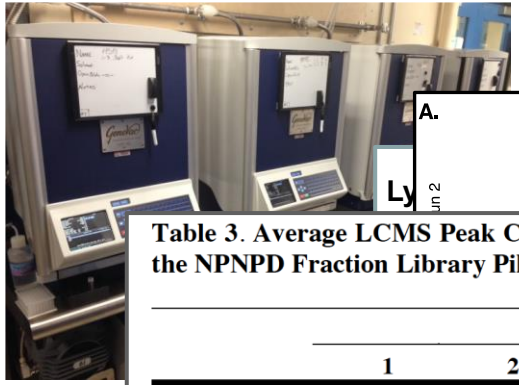
NPNPD Pre-fractionation Automation Goals

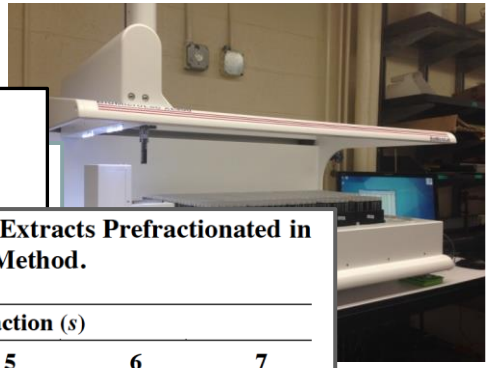






NPNPD Pre-fractionation Optimization and Automation





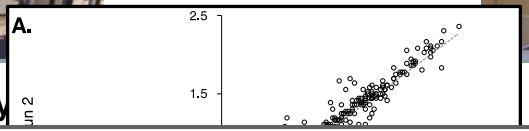
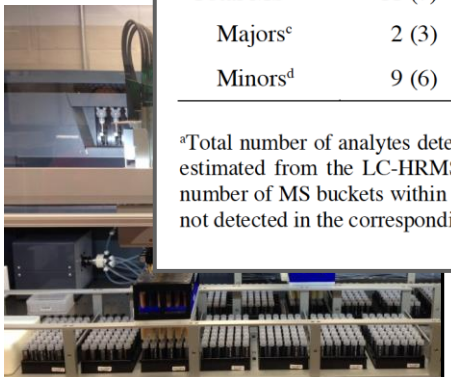
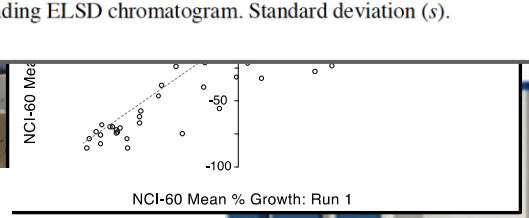


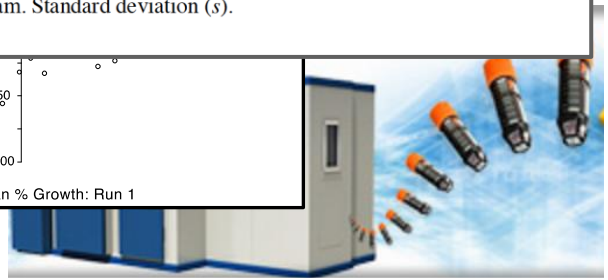
Table 3. Average LCMS Peak Count by Fraction for All of the Extracts Prefractionated in the NPNPD Fraction Library Pilot Study Using the C8, RP-2.7 Method.

	Avg Peak Count per C8 SPE Fraction (*s*)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
ELSD[a]	2 (1)	2 (2)	2 (2)	4 (3)	7 (3)	11 (8)	9 (8)
Total MS[b]	11 (7)	9 (8)	12 (8)	22 (16)	43 (44)	40 (34)	23 (12)
Majors[c]	2 (3)	2 (3)	3 (3)	7 (7)	17 (21)	12 (12)	6 (3)
Minors[d]	9 (6)	8 (5)	9 (6)	15 (13)	26 (25)	28 (27)	17 (11)

[a]Total number of analytes detected using an evaporative light scattering detector (ELSD). [b]Total number of analytes estimated from the LC-HRMS data and defined by *m/z* value, retention time and intensity (MS buckets). [c]Total number of MS buckets within each detectable ELSD retention time window. [d]Total number of MS buckets that were not detected in the corresponding ELSD chromatogram. Standard deviation (*s*).







NPNPD Pre-fractionation Progress

- >525,000 natural product fractions produced
- First 326,000 fractions released to the public
- ~175,000 new fractions to be released in Q1 2022
- >20,000,000 wells of fractions plated in 384-well plates for shipping and stored in repository
- >5,000,000 samples shipped to screening centers worldwide (>previous 40 years of NPB shipments combined)
- Initial publications on:
  - library and methods [Thornburg *et al.* ACS Chem. Biol. 2018]
  - use for screening [Wilson *et al.* Nat Prod. Rep. 2020]
- Adoption of NPNPD methods and automated systems by research groups in U.S. (MI, MS, VA, CA), S. Africa and Sweden.

Expand Chemical Diversity from Culturable Organisms

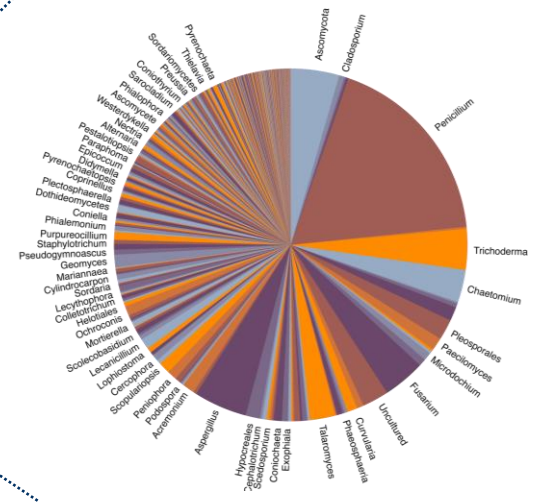
- 24,000 fungal samples supplied by the University of Oklahoma citizen science program.
- New fermentation facility at the NCI at Frederick
- New methods for increasing microbial extract production
- Provides renewable source of active molecules

Hypocrea sp.

Aspergillus fumigatus

Gibberella sp.

Tolypocladium sp.



Penicillium chrysogenum

Aspergillus versicolor

Hypocrea crassa

Penicillium striatisporum

Paecilomyces sp.

Monographella sp.

Image credits: NCI / DTP and Natural Products Support Group

Provide Pre-fractionated Library to Screening Centers

>70 requests from screening centers for NPNPD fractions, 40 MTA agreements completed,

Academia:

Harvard University
Scripps Research Institute
Dana Farber Cancer Institute
Howard Hughes Medical Institute
University of Pennsylvania
Yale School of Medicine
University of Michigan
University of Utah
University of Connecticut
Florida State University
Oregon State University
Brandeis University
University of California Santa Cruz
University of Florida
Arizona State University
Ohio State University
Vanderbilt University
Baylor College of Medicine
Florida A & M University
University of Wisconsin
Cornell University
Columbia University
Rutgers University
Jacksonville University
University of Texas, San Antonio
University of Iowa
University of Minnesota
University of California Dan Diego
Swinburne University (Sarawak)
Australia National University
University of Melbourne (Australia)
University of Kent (UK)
University of Leeds (UK)
University of Toronto (Canada)
Simon Fraser University (Canada)
University of Padua (Italy)

Industry:

Astra Zeneca
Corteva
LifeMine Therapeutics
Deinove (France)
JMI Laboratories
Enveda Biosciences

U.S. Government:

NCATS
DOD/WRAIR
NIAID
NCCIH
NCI/CCR

Encouraging High Throughput Screening Support

Participation from Other U.S. Government Entities to Date

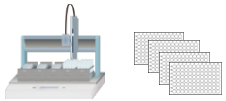
- NIAID - Screened the NPNPD pre-fractionated library against ESKAPE pathogens. New IAA for joint research being finalized.
- DOD - Funded research between WRAIR and the NCI to screen against malaria and leishmaniasis.
- NCCIH - Issued grant for a global natural product database of NMR data. Is funding NPNPD HEAL initiative efforts and IGNITE screening grants.
- NCATS - Screening NPNPD fraction library against a variety of targets. Is funding NPNPD efforts to isolate and identify active compounds as part of the NIH HEAL initiative.

Provide Faster Analytical Resources to Expedite Translation

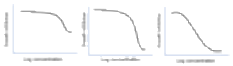
- Created an automated 2nd stage chromatography system that processes >500 samples, producing >10,000 highly pure sub-fractions, in 2 weeks
- Significantly improves speed and efficiency of “hit” confirmation by screening laboratories
- Generates valuable chemical information on identity of active compounds
- Reduces cost of natural product screening
- Conserves extracts (1 mg instead of 1g)

1. ASSAY

Generation of plates

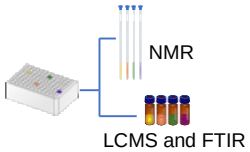


Assay data



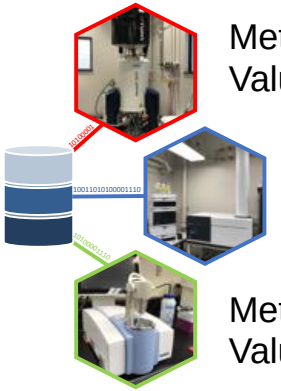
2. DEREPLICATION

Repeat plates



Analytical data on active wells

3. ACTIVE PRINCIPLES



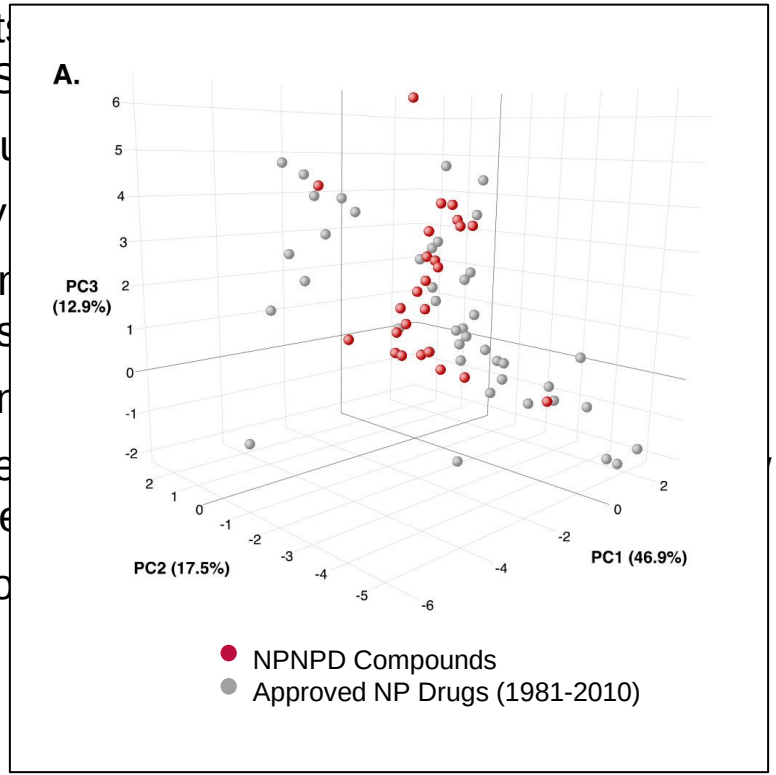
Method: **^{1}H NMR spectroscopy**
Value: High throughput, fast and comprehensive.

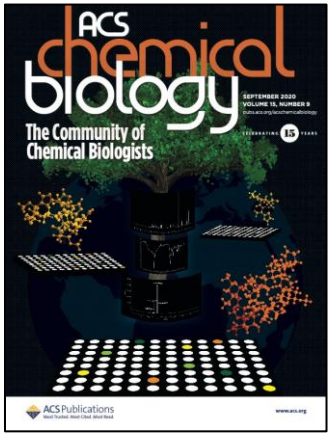
Method: **Mass spectrometry**
Value: Sensitive, high throughput.

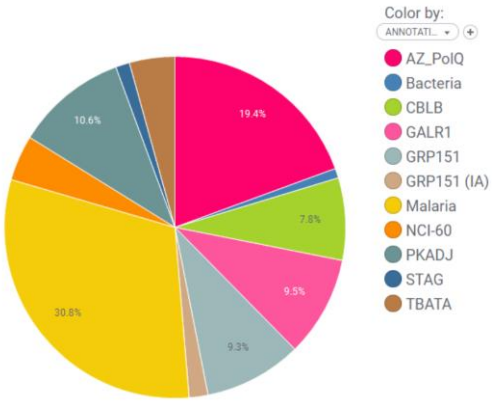
Method: **FTIR spectroscopy**
Value: Small footprint, large spectral range.

NPNPD High Throughput Isolation and Identification

- Completed project
  MTP/CCR, NCATS
- >65,000 purified su
- ~80-90% recovery
- ~70% of active cor
  single automated s
- Requires significar
- For ~80% of active
  activity in the same
- Results and metho
  Chem. Biol. 2020]

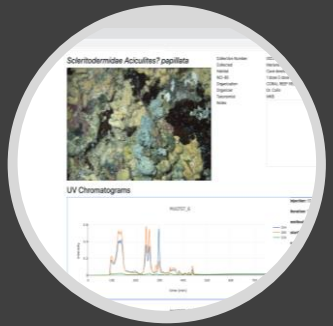


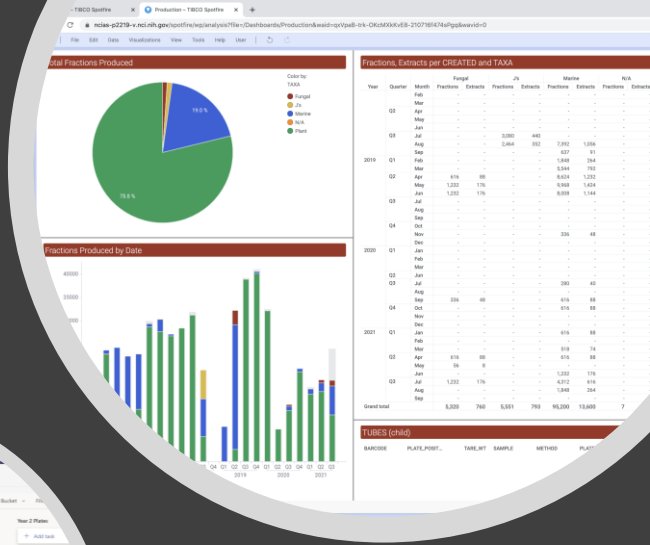


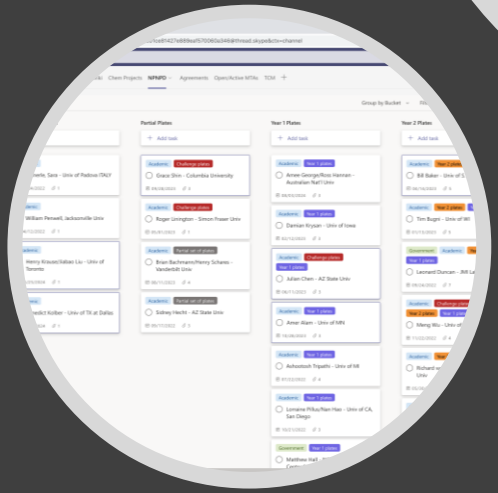


NPNPD Data Handling Systems

- Secure, web accessible monitoring and reporting
- Monitor primary fraction production, 384 well plate generation, per collaborator assay metrics
- Track progress of collaborations through MTA receipt, plate distribution, screening, 2nd stage chemistry
- Visualize geographic, photographic and taxonomic collection data
- Access chemical and biological data on specific source materials

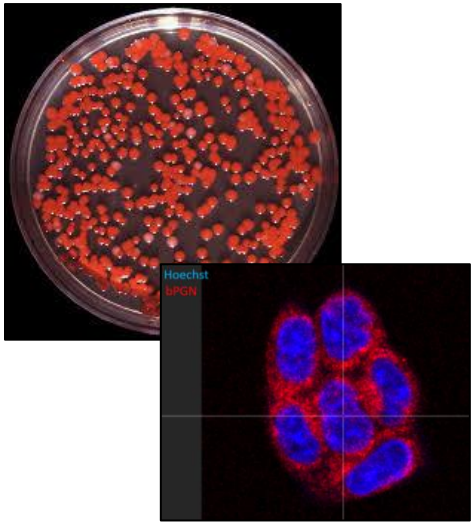


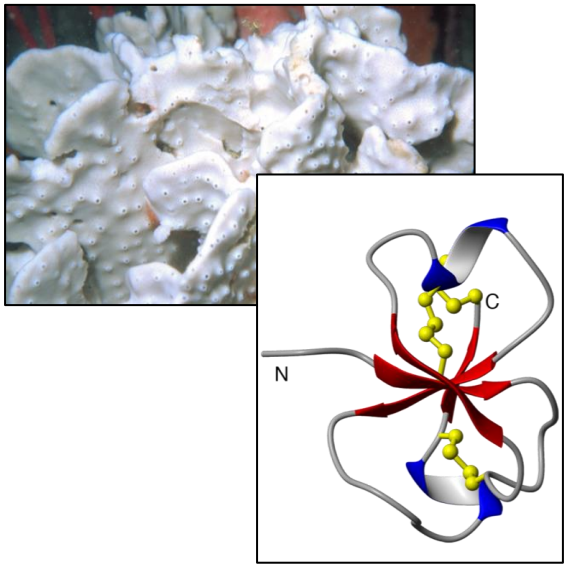


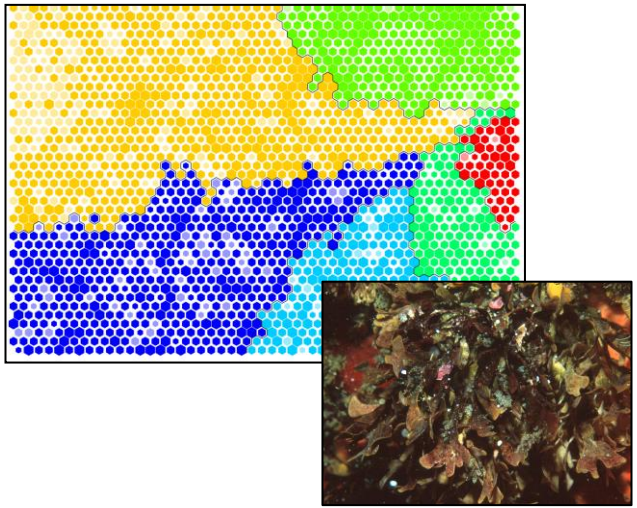


Recent Outcomes from NCI Natural Product Discovery Efforts

- DSF Screen for modulators of pre-miR-21 stability
- Identification of allosteric inhibitor of TDP-1
- Bioinformatic analysis to identify novel natural products

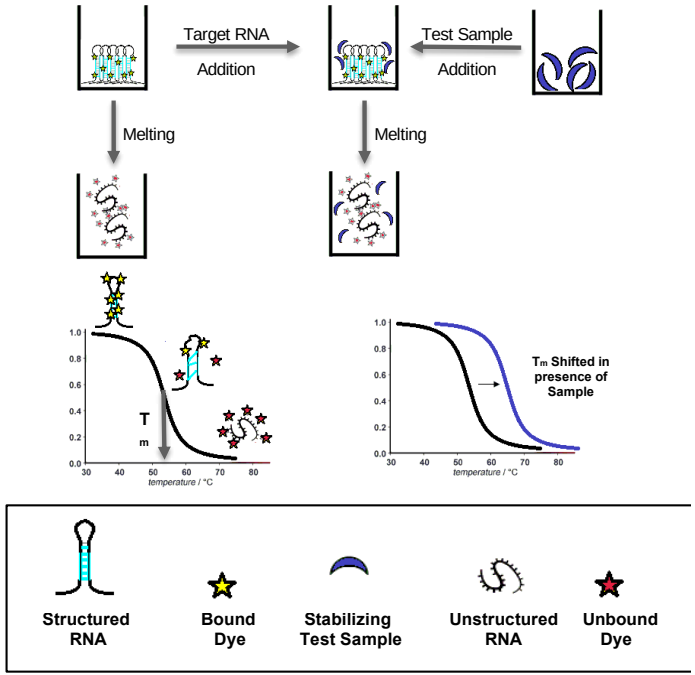




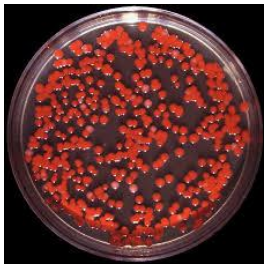


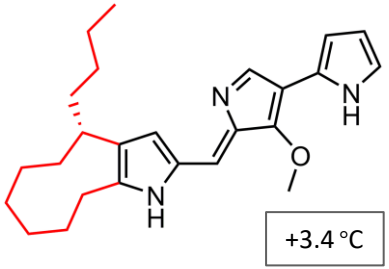
Biophysical Assays of Natural Products that Alter Macromolecular Stability

Basis of Differential Scanning Fluorimetry (DSF) Assay



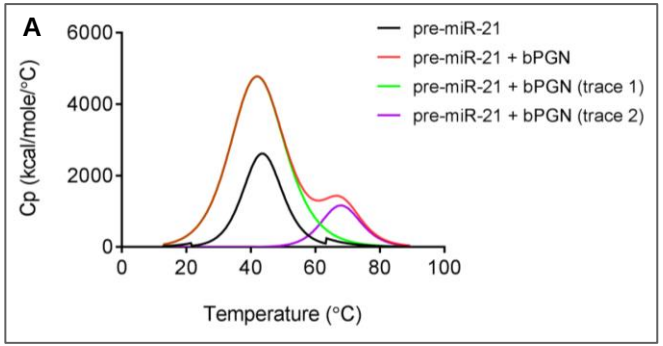
- DSF assay can detect both the stabilization and destabilization of macromolecular species
- Never used previously with natural product extract samples
- Reduced to practice in the MTP/CCR/NCI as a high-throughput assay
- Identified a natural product from *Serratia marcescens* that stabilizes pre-miR-21

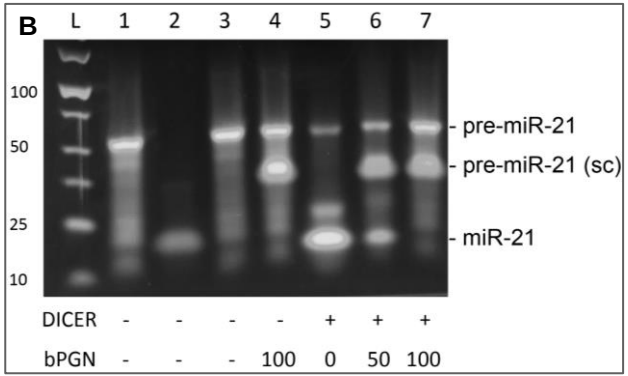


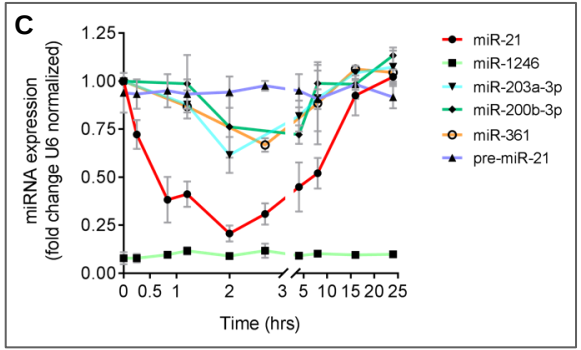


bPGN (1)

Discovery of pre-miR-21 Modulating Natural Products







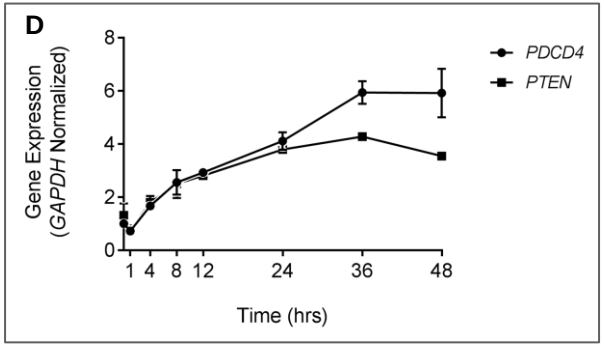
A. bPGN stabilizes pre-miR-21

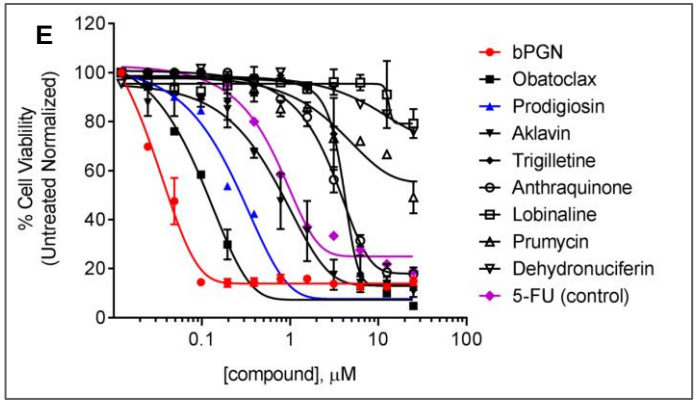
B. bPGN induces a second, supercoiled form of pre-miR-21

C. miR-21 levels are reduced in the cell

D. Reduction in miR-21 causes PDCD4 and PTEN levels to increase

E. HCT116 cells die as a result of treatment with bPGN

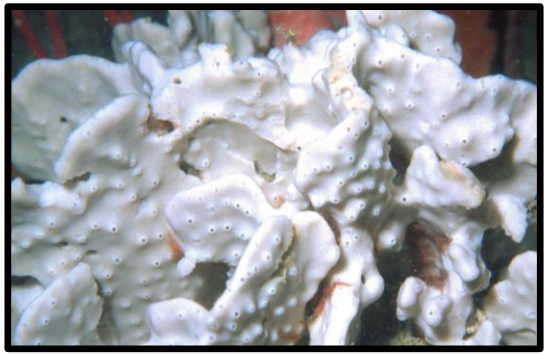




Matarlo, et al. *Cell Chem. Biol.* 2019.

Novel Peptide Inhibitor of Tyrosyl-DNA Phosphodiesterase 1

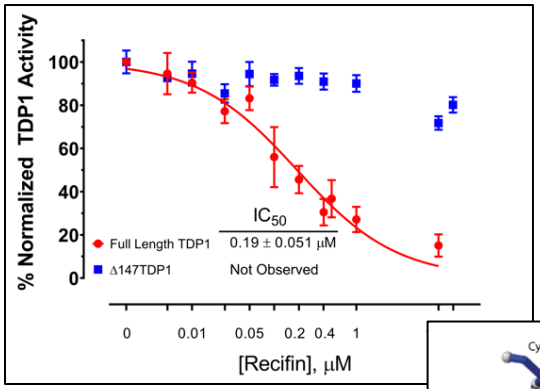
TDP1 Hydrolyzes bond between tyrosine of Topoisomerase 1 and 3' end of DNA and reverses TOP1 inhibition

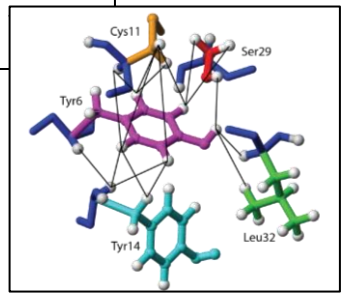


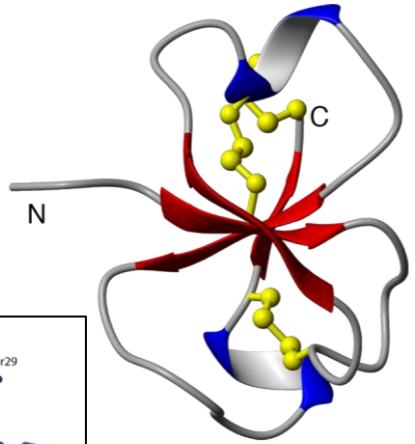
- Recifin A is a novel peptide from the sponge *Axinella* sp.
- It is the first allosteric inhibitor of Tdp1
- Is a completely new class of peptide structure distinct from cystine knot peptides
- "Tyr-Lock" peptides patented and are being licensed by the NCI.

Recifin A

eEAFCYSDRFCQNYIGSIPDCCFGRGSYSFELQPPPWECYQC



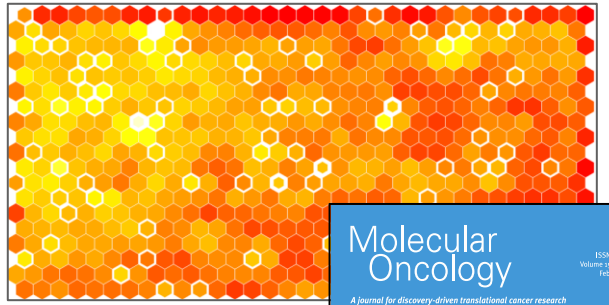


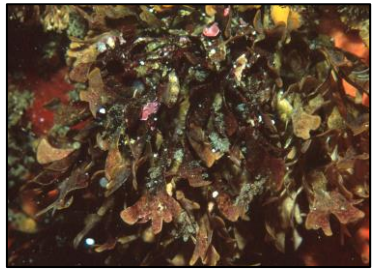


Krumpe, *et al*. *J. Am. Chem. Soc.* 2020.

Bioinformatic Tools to Drive Anti-cancer Drug Discovery

- Based on self-organizing-map analysis of NCI-60 cytotoxicity data developed as part of the NPNPD
- Initial project re-parsed NCI-60 activity profiles for >1400 natural products by the “genetic signature” of the cell lines to identify correlations with specific mutations/expression levels/SNPs etc.
- The methodology has now been used for ~140,000 records for natural products extracts to predict potential mechanisms for active extracts from NCI-60 data files
- NPB has isolated novel natural products, with new carbon skeletons, that selectively target Schlafen 11 overexpressing cells, from biometrically-selected extracts (*i.e.* bryozoan)





Krushkal *et al. Mol. Oncology, 2021*

Current Status and Future Prospects for the NPNPD

NPNPD Is becoming a central hub for natural products research

- New Technologies and Methods - increased throughput, reduced costs
- New Chemical Diversity – pre-fractionated library, culturable organisms
- New Partnerships – other NIH institutes, extramural research groups
- New Bioactive Compounds – new assays, activities and chemical structures

One possible option to build upon NPNPD foundational technologies and resources

- NCI grants for screening the NPNPD library – IGNITE Program
  - Innovation Grants to Nurture Initial Translational Efforts
  - R61/R33 two step funding mechanism to encourage HTS assay development and screening

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